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Exposures of Healthy and Asthmatic Volunteers to Concentrated Ambient Ultrafine Particles in Los Angeles

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Adult volunteers (17 healthy, 14 asthmatic) were exposed in a controlled environmental chamber to concentrated ultrafine particles (UFP) collected in a Los Angeles suburb with substantial motor vehicle pollution. Exposures lasted 2 h with intermittent exercise. Inhaled particle counts (mean 145,000/cm^3, range 39,000–312,000) were typically 7–8 times higher than ambient levels. Mass concentrations (mean 100 μg/m^3, range 13–277) were not highly correlated with counts. Volunteers were evaluated for lung function, symptoms, exhaled nitric oxide (eNO), Holter electrocardiography, and inflammatory markers in peripheral blood and induced sputum. Relative to control (filtered air) studies, UFP exposures were associated with a 0.5% mean fall in arterial O_2 saturation estimated by pulse oximetry ($p < .01$), a 2% mean fall in forced expired volume in 1 sec (FEV_1) the morning after exposure ($p < .05$), and a transient slight decrease in low-frequency (sympathetic) power in Holter recordings during quiet rest ($p < .05$). Healthy and asthmatic subjects were not significantly different across most endpoints. Thus, this initial experimental study of human volunteers exposed to concentrated Los Angeles area ambient UFP showed some acute deleterious cardiopulmonary responses, which, although generally small and equivocal as in previous studies of larger sized concentrated ambient particles, might help to explain reported adverse health effects associated with urban particulate pollution.

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Acute and chronic exposures to particulate matter (PM) in urban air pollution have been associated with illness and premature death in numerous epidemiologic studies. The association is widely, but not universally, accepted as causal (American Thoracic Society, 1996; Vedal, 1997; Dockery, 2001; Green & Armstrong, 2003; Englert, 2004; Schulz et al., 2005; Pope & Dockery, 2006). Urban PM may be classified into three size ranges. The ultrafine or condensation mode (defined as particles smaller than 0.15 μm in aerodynamic diameter) and the fine or accumulation mode (0.15 to 2.5 μm) derive mostly from combustion processes, while the coarse mode (above 2.5 μm) derives mostly from soil and mechanical processes. Ultrafine particles (UFP) usually contribute only a small fraction of urban PM mass, but account for the largest share of particle number and surface area, which may significantly influence their toxicity (Hinds, 1999).

Thus far, it has been difficult to find consistent evidence of specific physical properties, chemical properties, or source characteristics that contribute to acute toxicity of urban PM (Schlesinger et al., 2006). Yet theoretical considerations, along with recent toxicologic and epidemiologic evidence, point to UFP as an important contributor (Kreyling et al., 2004; Oberdörster et al., 2005; Schulz et al., 2005; Delfino et al., 2005; Sioutas et al., 2005; Donaldson et al., 2005). Potentially important features of UFP include efficient respiratory-tract deposition, large surface area for catalytic interactions with biological substrates or adsorption/delivery of toxic gases, and ability to translocate into other susceptible organs including the heart and brain. UFP in typical urban PM contain transition metals and oxygenated organic compounds likely to present oxidative stress to biological systems. Furthermore, their ambient outdoor concentrations may be markedly elevated on or near heavily traveled roadways, where many urban dwellers are exposed for extended periods (Zhu et al., 2002a, 2002b; Westerdahl et al., 2005).

Schulz et al. (2005), surveying the current evidence for human health effects of combustion-derived PM, find support for three distinct types of mechanisms mediating cardiovascular effects: (1) pulmonary and/or systemic inflammatory responses inducing endothelial dysfunction, a pro-thrombotic state and promotion of atherosclerosis, (2) dysfunction of the autonomic nervous system in response to direct reflexes from receptors in the lungs and/or to local or systemic inflammatory stimuli, and (3) cardiac malfunction due to ischemia and/or altered ion-channel functions in myocardial cells. They conclude that "available data are consistent with the occurrence of a systemic inflammatory response and an alteration of autonomic cardiac control, but evidence of endothelial dysfunction, pro-coagulatory states, and PM-related myocardial malfunction is as yet scarce." One important source of mechanistic evidence is studies of animals and humans exposed to concentrated ambient particles that use virtual impactors to entrain most particles from a large flow of air within a smaller flow that passes through an exposure chamber. Human volunteers, both healthy and with chronic respiratory diseases, have been studied repeatedly with fine particles at concentrations representing severe ambient pollution episodes; see for example Gong et al. (2003a, 2003b, 2004a), Devlin et al. (2003), and Brook et al. (2002). Although no clear pattern has emerged, most of these studies have shown small statistically significant changes in cardiovascular physiology or blood biochemistry consistent with one or more of the aforementioned mechanisms. No meaningful changes in lung function have been found with these exposures, typically of 2 h duration with intermittent exercise. UFP, although present in the experimental exposure atmospheres, were not always measured. UFP are not concentrated efficiently by fine-particle concentrators, and not removed efficiently by typical filter media. Thus, it is not likely that UFP differed markedly between exposure and control conditions, or contributed meaningfully to effects found in concentrated-fine-particle exposures.

Most prior experimental human exposures to UFP have employed artificially generated, single-substance particles at concentrations above the usual ambient range. Frampton et al. (2004, 2006) exposed volunteers to ultrafine carbon particles at mass concentrations of 10 or 25 $\mu g/m^3$, and found subtle changes in peripheral blood leukocyte distribution and adhesion molecule expression, suggesting effects on vascular endothelial function. They found no measurable effects on systemic inflammation, coagulation properties, or pulmonary function tests. Beckett et al. (2005) compared effects of zinc oxide particles in the fine and ultrafine size ranges, at 500 $\mu g/m^3$. Measures of leukocyte surface markers, hemostasis, cardiac electrophysiology, airway inflammation (as measured from induced sputum), or pulmonary function did not change with either size. Kuschner et al. (1997) exposed volunteers to a mixture of fine and ultrafine magnesium oxide particles from a model furnace. They found no significant effects on cell and cytokine concentrations (determined by bronchoalveolar lavage), peripheral blood neutrophil concentrations, or pulmonary function.

A preliminary report on initial experimental exposures of volunteers to concentrated ultrafine ambient particles in Chapel Hill, NC, indicated no detectable effects on most measures of pulmonary function, airway or systemic inflammation, cardiac electrophysiology, or blood coagulability, although a small significant increase in plasma D-dimer concentration and a small significant decrease in one measure of heart-rate variability were found (Samet et al., 2007).

This article presents results from the first investigation in the heavily polluted metropolitan Los Angeles area employing a large-scale ultrafine particle concentrator and human subjects. Healthy adults and atopic adult asthmatics with mild disease were exposed in a controlled chamber to concentrated UFP collected from a location heavily impacted by motor vehicle pollution, but not immediately adjacent to heavy traffic. Concentrations during the 2-h experimental exposures were typically 7 to 8 times ambient, but still well within the range observed in freeway traffic (Westerdahl et al., 2005). Health responses were evaluated via symptom questionnaires, pulmonary function

tests, Holter electrocardiogram (ECG) recordings during and after exposure, and probes of airway inflammation—periodic measurements of exhaled nitric oxide (eNO), and analyses of sputum induced 20–24 h after exposure. Biochemical measurements of inflammatory mediators in peripheral blood were also obtained, although not all species could be assayed in all subjects.

METHODS

Exposure Facility and Protocol

The UFP exposure system was located at the Los Amigos Research and Education Institute in Downey, California. Its basic design has been described previously (Kim et al., 2000; Misra et al., 2004). In brief, UFP are concentrated by passing the ambient aerosol successively through a saturator where particles are mixed with ultrapure warm water vapor, then a cool condenser where particles grow into the fine size range due to water condensation as a result of supersaturation, then a virtual impactor, and finally a diffusion dryer where particles lose water and return to their original size. Relatively little loss or alteration of particles occurs during this process (Sioutas et al., 1999; Kim et al., 2000). The current system is scaled up from concentrator systems used previously in rodent toxicologic studies (Kleinman et al., 2005; Cassee et al., 2005; Kooter et al., 2006). It was interfaced to a single-person exposure chamber, as described in a previous report of coarse-particle exposures (Gong et al., 2004b). This was located about 1.5 km from an 8-lane freeway carrying many diesel trucks, and 100 m from a 6-lane street. Ambient air was sampled at 1200 L/min about 1 m above roof level, 5 m above ground level. Using 12 parallel condenser/impactor/dryer units, a minor flow of 60 L/min, containing most of the ambient particles, was diverted to the exposure chamber, first passing through a slit impactor (Misra et al., 2002) to remove most particles above 0.18 μm aerodynamic diameter. A total flow of 150–170 L/min was drawn from the rear of the exposure chamber by a conventional air pump. Under ordinary operating conditions the concentrator system could increase the particle count in inhaled air to 7–8 times the ambient level. (The ideal enrichment factor is 8, which is the ratio of the normal intake to concentrated flows, 1200/150.) Inlet air to make up the difference between total flow and concentrator output was cleaned by a HEPA filter with 99.9% efficiency for 0.3-μm particles, and delivered to the front of the chamber separate from the concentrator flow. Under control conditions, the concentrator minor flow was shut off and only filtered air was delivered. The first 7 subjects underwent whole-body exposures to concentrated UFP. Subsequently, to improve control of inhaled concentrations, exposures were performed via a facemask system modeled after that of Brook et al. (2002). No CO_2 scrubber was used because overall air exchange was sufficient to control CO_2.

Particle count was measured in real time by a condensation particle counter (CPC) model 3022A (TSI Inc., St. Paul, MN) sampling from the exposure chamber inlet. To estimate the concurrent ambient particle count and concentrator efficiency, the CPC sampling line was briefly moved to sample outdoor air once just before beginning the exposure, once near its midpoint, and once just after it finished. In whole-body exposures, time-integrated samples of particulate matter were collected by low-volume sampling (5 L/min) at the rear of the chamber on Teflon and quartz filters. These samples, unlike the CPC particle count, would include particles generated by the subject or the exercise cycle. Subjects wore lint-free scrub suits plus head and shoe covers to minimize particle generation. In exposures via mask, Teflon and quartz filter samples were collected from the chamber inlet at 2.5 L/min. The switch to mask exposures resulted in significantly higher particle counts but lower mass measurements in concentrated UFP exposures, as well as significantly lower particle counts and nonsignificant decrease in mass measured in control studies. However, there was considerable overlap of exposure data distributions between whole-body and mask exposures, and preliminary statistical analyses of health endpoints did not show significant differences between them. Accordingly, whole-body and mask results were pooled for the final analysis. Teflon filters were weighed at standardized temperature and relative humidity to determine particle mass concentrations, and sent to Chester LabNet Inc., Tigard, OR, for elemental analysis by x-ray fluorescence (XRF). Quartz filters were sent to the Southern California Particle Center and Supersite analytical laboratories at University of California, Los Angeles, for analysis of elemental and organic carbon. To estimate ambient exposures in proximity to experimental exposures, concentrations of particles below 10 μm diameter (PM_{10}), from the California Air Resources Board online database, were averaged between the two monitoring stations nearest the laboratory over the 24 h prior to the beginning of exposure.

Normally, each subject was exposed once to filtered air alone and once to concentrated UFP, in randomized order, with 2 wk or more between exposures. On rare occasions where the UFP concentration was low due to unfavorable ambient conditions, data were discarded and the UFP exposure was repeated at a later date.

Details of the exposure protocol and response measurements have been described previously (Gong et al., 2003a, 2003b, 2004a, 2004b). Each 2-h exposure began near 9 a.m. The subject exercised for 15 min of each half hour at a ventilation rate of 15–20 L/min per square meter of body surface. Conditions were considered single-blind, because the concentrator and real-time monitor were unavoidably visible to personnel measuring health responses, although they were not informed of exposure conditions.

Routine health measurements were performed just before exposure ("pre"), just after exposure ("post"), 4 h after exposure ended ("hour 4"), and the following morning about 22 h after exposure ended ("day 2"). These included symptoms (recorded by standardized questionnaire), blood pressure measured by an electronic sphygmomanometer (Sunbeam-Oster, Hattiesburg, MS), lung function (forced expiratory spirometry performed using a VMax 229 system, Viasys Inc., Yorba Linda, CA), arterial

oxygen saturation measured by fingertip pulse oximeter (N-3000, Nellcor Inc., Pleasanton, CA), and exhaled nitric oxide (eNO), measured online at 50 ml/s expiratory flow by standard procedures (American Thoracic Society, 1999), using a Sievers 280i chemiluminescent analyzer (GE Analytical Instruments, Boulder, CO). Inhaled NO concentration was recorded with the same analyzer; no attempt was made to remove NO from inhaled air. Venous blood was collected at pre, hour 4, and day 2. Frozen serum aliquots were shipped to the U.S. EPA National Health and Environmental Effects Research Laboratory (NHEERL), Research Triangle Park, NC, for assay of inflammatory mediators by enzyme-linked immunosorbent assay (ELISA) (Ghio et al., 2003; Yeatts et al., 2007). This work was donated, subject to other concurrent responsibilities of the laboratory; thus, not all assays could be performed for all subjects (see Results). Sputum induction was performed on day 2 only, after completion of other testing and prophylactic administration of a bronchodilator, by the methods of Fahy et al. (1995). Total and differential cell counts were performed on site, and frozen aliquots were shipped to the Center for Environmental Medicine, Asthma and Lung Biology, University of North Carolina, Chapel Hill, for biochemical analysis. An initial Holter ECG recording ("Holter period 1") was started just before the preexposure testing session and continued through hour 4 testing (about 7 h total). Another recording ("Holter period 2") was started immediately thereafter and continued through day 2 testing. Mortara H-12 Holter recorders (Mortara Instruments, Milwaukee, WI) were employed. Data cards were sent to NHEERL for analysis. Additional details of health measurements are given in the Results section.

Subject Recruitment and Screening

As in previous studies with larger particles (Gong et al., 2003a, 2003b, 2004b), nonsmoking healthy and asthmatic adults aged 18–50 yr were recruited by advertisements and invitations to prior participants, and medically screened to verify their asthma status and rule out medical contraindications. Asthmatics were selected as the first potentially "extra-susceptible" group to be studied both for ethical reasons—they were considered less likely to experience clinically untoward effects than other groups with chronic disease—and for scientific reasons. Asthmatics are expected to have elevated levels of preexisting airways inflammation, and modified innate host defense capabilities, which importantly include suboptimal antioxidant defense capability (Kongerud et al., 2003). They have shown increased deposition of inhaled UFP, compared to healthy subjects (Chalupa et al., 2004) . There also appears to be an association between asthma and cardiovascular illness (e.g., Soriano et al., 2005), and circumstantial evidence that this may relate to airway inflammation (Suissa et al., 2003).

Table 1 summarizes subject characteristics. Asthmatics had mild intermittent or mild persistent asthma (National Asthma Education Program, 1997) and were not using corticosteroids or leukotriene modifiers. Those using short-acting bronchodilator medications were instructed to withhold them for 6 h prior to each experimental exposure. All asthmatics and two healthy subjects were atopic by history. Allergy skin tests were not available for all subjects, so more detailed characterization was not feasible. All subjects gave written informed consent to participation. The protocol and consent form were reviewed and approved by the local Institutional Review Board.

TABLE 1
Subject characteristics

Characteristic	Healthy	Asthmatic
Number, gender	17 (12 F, 5 M)	14 (5 F, 9 M)
Age (mean ± SD)	24 ± 8	34 ± 12
Height, cm (mean ± SD)	183 ± 5 (M)	176 ± 7 (M)
	166 ± 8 (F)	169 ± 5 (F)
Weight, kg (mean ± SD)	89 ± 19 (M)	81 ± 20 (M)
	63 ± 8 (F)	88 ± 27 (F)
FEV_1, % predicted[a] (mean ± SD)	107 ± 10	87 ± 16
Ethnic group[b]	7 H, 10 W	2 A, 4 B, 3 H, 5 W

[a]Morris et al. (1971), adjusted for ethnic group as appropriate.
[b]A = Asian, B = Black, H = Hispanic, W = White not Hispanic.

Data Analysis

Analyses of variance (ANOVA) with repeated measures on subjects were performed initially to test for main and interactive effects of clinical group (healthy, asthmatic), atmosphere (filtered air, UFP) and time (pre, post, hour 4, day 2) on each health-response variable. A significant atmosphere–time interaction would indicate an effect of UFP. Alternatively, a "net change attributable to UFP exposure" was calculated for each health response variable and time of measurement after exposure, e.g., $(\text{post} - \text{pre})_{\text{UFP}} - (\text{post} - \text{pre})_{\text{filtered}}$. Each net change was tested for significant difference from zero by its t-statistic, and net changes were compared among post, hour 4, and day 2 measurements by ANOVA. For variables with some missing or unsatisfactory measurements, repeated-measures ANOVA for unbalanced designs was employed, with maximum-likelihood estimation of missing values. Because individuals' exposure concentrations varied markedly, correlation and regression analyses were performed to test for significant exposure-response relationships, i.e., associations between higher exposure levels and more unfavorable net changes. (Given the small sample size, these tests would have low power to reject the null hypothesis.) Exposure measures used were particle count, mass concentration, concentrations of specific elements found meaningful by factor analysis (see Results), chamber temperature, and past 24-h-average ambient PM_{10}. Statistical differences were considered significant at $p < .05$. No adjustment was made for multiple

TABLE 2
Atmospheric measurements in exposure chamber, and associated ambient air measurements (mean ± SD)

	Filtered air control		Ultrafine particle exposure	
Parameter	Mean ± SD	Range	Mean ± SD	Range
Particles/cm^3	320 ± 630	1–3570	143,000 ± 60,000	39,000–312,000
Mass, μg/m^3	13 ± 12	0–50	100 ± 68	13–277
Temperature, °C	22 ± 1	19–25	23 ± 2	20–27
Relative humidity, %	59 ± 10	42–88	67 ± 11	40–88
Elemental carbon, μg/m^3	0.3 ± 0.7	0–2.4	7.7 ± 7.0	0–24.6
Sulfur, μg/m^3	0.1 ± 0.2	0–1.3	3.2 ± 2.2	0.03–8.6
Silicon, μg/m^3	0.3 ± 0.3	0–0.9	1.3 ± 0.9	0–3.5
Iron, μg/m^3	0.1 ± 0.1	0–0.5	0.9 ± 1.1	0–4.3
Aluminum, μg/m^3	0.2 ± 0.2	0–0.6	0.5 ± 0.5	0–1.6
Calcium, μg/m^3	0.2 ± 0.3	0–1.3	0.5 ± 0.4	0.02–1.6
Ambient particles/ml	17,900 ± 11,600	1050–46,500	20,200 ± 10,500	6100–46,200
Ambient temperature, °C	23 ± 5	12–33	22 ± 5	13–30
Ambt. relative humidity, %	48 ± 13	26–93	48 ± 15	25–98
Station[a] PM_{10}, μg/m^3	32 ± 9	21–61	32 ± 15	7–102

[a]Mean for 24 h prior to start of exposure, from 2 ambient monitoring stations closest to laboratory (Long Beach and Downtown Los Angeles).

statistical tests; thus, given the large number of tests, a few results should appear "significant" by chance alone.

RESULTS

Exposures

Table 2 summarizes air monitoring results from all exposures. In filtered air, generally low particle counts indicated effective removal of ambient UFP. Appreciable mass concentrations in the initial whole-body exposures (mean 18 μg/m^3) were attributable to larger particles released from subjects and exercise equipment. Lower but still appreciable concentrations in the subsequent mask exposures (mean 11 μg/m^3) were unexpected. One possible explanation is that particles from the subject's exhalate or facial surface penetrated the relatively short distance upstream from the mask to the filter sampling port. Particle counts during UFP exposures averaged about 7 times higher than concurrent outdoor ambient levels. Individual UFP exposure particle counts, which varied by as much as a factor of 8, showed a relationship with concurrent ambient particle counts ($r^2 = .50$), but not with exposure PM mass concentration, ambient temperature, chamber temperature, or past 24 hr ambient PM_{10} concentration ($r^2 \leq .12$). The substantial mass concentrations in most UFP exposures reflect the fact that much of the relevant ambient particulate matter in Los Angeles falls near the upper end of the ultrafine size range—exceeding 0.1 μm—and therefore is comparatively massive (Sardar et al., 2005). Temperature, relative humidity, and past 24-h ambient PM_{10} were not different between filtered air and UFP exposures. However, the chamber temperature increment in UFP relative to filtered air—about 1°C on average—was significant ($p < .01$) despite no corresponding difference in ambient temperature. This is attributable to heat generated by the UFP concentrator and limited air-conditioning capacity in the laboratory. Among specific particle components measured, elemental carbon and sulfur were markedly increased in UFP and iron was also increased substantially, relative to filtered air. Silicon, calcium, aluminum, and sodium increased only modestly in UFP, suggesting that internally generated particles were an important source of these elements. Organic carbon measurements (data not shown) were implausibly high in filtered air, only slightly lower than in UFP, indicating a positive measurement artifact as observed in previous concentrator studies (Gong et al., 2003a, 2003b, 2004a, 2004b).

Factor analysis was performed on UFP exposure variables including particle count, mass concentration, elemental carbon, iron, sulfur, aluminum, silicon, calcium, and sodium, the latter being the most abundant elements found by x-ray fluorescence. Three meaningful factors were found. Soil-associated elements—silicon, aluminum, calcium, and iron—were strongly loaded on the first factor, which explained the largest proportion of variance. Elemental carbon and particle count were strongly loaded on the second factor, and sulfur on the third. Based on these results, sodium, iron, elemental carbon, and sulfur were selected as independent variables to be tested in exposure-response analyses, along with particle count and total mass concentration.

Responses

Spirometry

Forced expired volume in 1 s (FEV_1) showed a significantly ($p < .05$) time-varying pattern of net changes after UFP

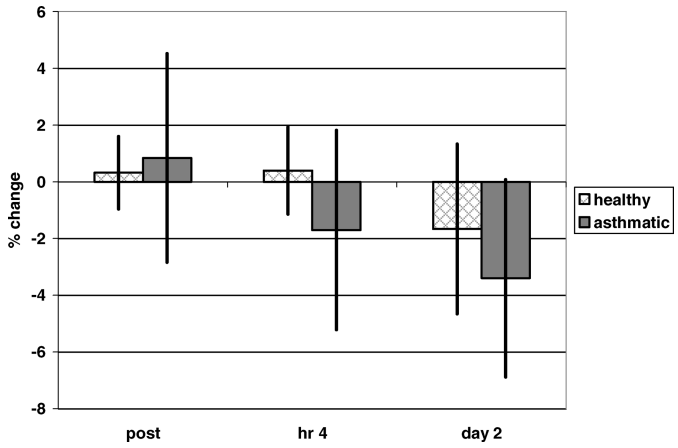

FIG. 1. Net change of forced expired volume in 1 s (FEV_1) associated with ultrafine particle exposure, as percentage of baseline measurement (prefiltered air). Wide vertical bar indicates mean; narrow bar indicates 95% confidence limit.

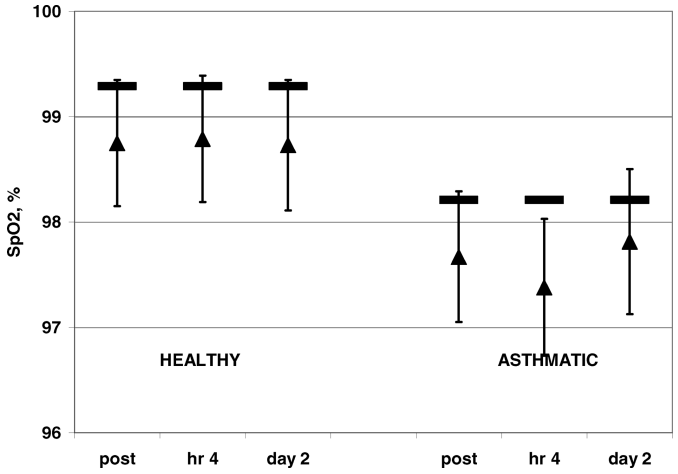

FIG. 2. Arterial oxygen saturation as estimated by pulse oximetry (SpO_2) in relation to ultrafine particle exposure. Horizontal bars indicate mean baseline measurements; diamonds indicate mean net change relative to baseline; vertical flags indicate 95% confidence limits of mean change.

exposure. Figure 1 shows the mean net changes as percentages of baseline (pre-filtered-air) FEV_1. There was a minimal increase immediately postexposure, a minimal decrease at hour 4, and a more negative change on day 2, which was still small (averaging 2.4% for all subjects, comparable to usual test-to-test variability for an individual). The overall mean net change across all 3 time points was not significantly different from zero. Although their respective mean values suggested a larger loss and earlier onset in asthmatics compared with normals, the group difference was nonsignificant. Forced vital capacity and maximal mid-expiratory flow showed similar patterns of net changes that did not reach statistical significance. No significant exposure-response relationships were found between spirometric variables and the aforementioned key exposure variables.

Pulse Oximetry

Baseline SpO_2 (percentage of oxygenated hemoglobin in arterial blood, as estimated by fingertip pulse oximeter) averaged 99.3% in healthy and 98.2% in asthmatic subjects. That difference was significant, $p < .01$. About 7% of SpO_2 data after exposure were missing, so maximum likelihood estimation was used to obtain the following results for all subjects. Net change after UFP exposure, averaged across all 3 times of measurement, was −0.55%, with standard error 0.18% ($p < .005$). This net loss in SpO_2 did not vary significantly across time, or between healthy and asthmatic subjects. The loss was still significant ($p < .05$) in the healthy and asthmatic groups analyzed separately. Figure 2 shows the mean net changes at each time point for each group. For 13 healthy and 9 asthmatic subjects with complete data, conventional ANOVA again showed a significant negative change (overall mean −0.46%, $p < .05$), with no significant differences by time or clinical group. No significant exposure-response relationships were found for SpO_2.

Exhaled Nitric Oxide (eNO)

Nitric oxide measurements were available for 14 healthy and 13 asthmatic subjects. Data were skewed, and so were log-transformed for analysis. Geometric mean baseline eNO was 20 ppb in healthy and 32 ppb in asthmatic subjects; that difference was nonsignificant. After UFP exposure, the overall mean net change was not significantly different from zero; however, eNO increased in healthy subjects but decreased in asthmatics, giving rise to a statistically significant interaction of atmosphere, time, and asthma status (Table 3). There was also significant variation by time: eNO tended to rise on day 2 compared to earlier measurements, regardless of atmosphere. Inclusion of inhaled NO concentration as a covariate did not change the statistical conclusions. No significant exposure-response relationships were found for eNO except for a negative relationship between sodium concentration and eNO net change. This attained significance only at the hour 4 measurement, with all subjects pooled.

Sputum

Total sputum cell counts were obtained for all 31 subjects. Satisfactory differential counts (from 3 slides with >50% viability and <40% squamous cells, 500 nonsquamous cells counted per slide) were obtained for 17 healthy and 10 asthmatic subjects. Table 4 summarizes results. Asthmatic and healthy subjects were not significantly different in their total cell counts, but asthmatics had fewer macrophages/monocytes and more eosinophils and lymphocytes. (Lymphocyte data were analyzed by nonparametric tests because distributions were unsuitable for ANOVA.) There were no significant differences in total cell counts between filtered air and UFP. There was a suggestion that

TABLE 3
Exhaled nitric oxide (ppb) mean values and ANOVA results

		Mean log(eNO)			Geometric mean eNO		
Atmosphere	Time	Healthy	Asthmatic	All	Healthy	Asthmatic	All
Filtered	Pre	1.297	1.510	1.400	19.8	32.4	25.1
	Post	1.278	1.507	1.388	19.0	32.1	24.4
	hr 4	1.265	1.516	1.386	18.4	32.8	24.3
	Day 2	1.289	1.593	1.436	19.5	39.2	27.3
UFP	Pre	1.253	1.519	1.381	17.9	33.0	24.0
	Post	1.311	1.490	1.397	20.5	30.9	25.0
	hr 4	1.269	1.488	1.375	18.6	30.8	23.7
	Day 2	1.320	1.541	1.427	20.9	34.8	26.7

Analysis of variance results

Factor	*F*	*p*
Asthma	3.2	0.0858
UFP	0.11	0.7398
UFP × Asthma	0.36	0.5545
Time	4.58	0.0078
Time × Asthma	1.26	0.2944
UFP × Time	0.64	0.5616
UFP × Time × Asthma	4.24	0.013

UFP exposure increased the percentage of neutrophils in healthy subjects but decreased it in asthmatics (atmosphere–asthma interaction $p < .1$), reminiscent of the contrasting changes in eNO. However, no significant correlation was found between individuals' net changes in eNO and sputum cell counts.

Sputum samples adequate for biochemical analysis were obtained from 16 healthy and 13 asthmatic subjects. The effects of processing the sputum samples with DTT on biochemical components were accounted for in the analysis as the values reported here were taken from standard curves that included the presence of DTT. Nonparametric statistical tests were employed with the resulting data, due to markedly non-normal distributions. Results are summarized in Table 4. Interleukins (IL) 6 and 8 and granulocyte/macrophage colony-stimulating factor (GMCSF) were quantifiable in nearly all samples; while IL 4, 10, and 13, interferon, and tumor necrosis factor alpha (TNF) were undetectable in many samples. None of these substances showed significant overall differences between UFP and filtered-air exposures, in analyses pooling all subjects. For IL-6, IL-8, and GMCSF, healthy and asthmatic groups were compared in terms of their differences between UFP and filtered air. Group differences were nonsignificant. Other variables were not compared in that manner because the many zero (nondetectable) results severely limited statistical power.

Table 5 summarizes significant ($p < .05$) correlations between individual exposure measures and sputum measures, both expressed as differences UFP – filtered air. These were calculated for all subjects pooled, and for healthy and asthmatic groups separately (cell counts, IL-6, IL-8, and GMCSF only). The number of significant relationships found was close to what would be expected by chance, and they showed no obvious pattern.

Symptoms

Most subjects reported either no symptoms, or one to two very mild symptoms, before exposure. The mean baseline total symptom score (before filtered air) was 1.0 overall, 0.5 in healthy, and 1.6 in asthmatic subjects. (The total score at any given time was obtained by scoring each of 18 different symptoms from 0 = absent to 5 = incapacitating, and then calculating the sum. Subtotals for respiratory symptoms, nonrespiratory chest symptoms [i.e., possible cardiac symptoms], and miscellaneous nonspecific symptoms were also calculated.) The overall mean total score increased by 2 points, indicating a moderate increase in one or a slight increase in two symptoms, during and immediately after UFP and filtered-air exposures, then decreased toward the baseline. This time variation was significant ($p < .005$). There were no significant differences between filtered air and UFP, nor between healthy and asthmatic subjects, in total scores or subtotals. However, net changes in subtotals as well as the total score showed significant ($p < .05$) positive relationships with particle number concentration during UFP exposure. That is, subjects whose UFP exposures had relatively high particle counts tended to report more symptoms with UFP than with filtered air, while subjects with low UFP particle counts

TABLE 4
Sputum assays: Mean values, summary of conventional ANOVA or nonparametric statistical test results

	Healthy		Asthmatic		
Parameter	Filtered	UFP	Filtered	UFP	Note
Total cell counts([thousands/ml)					
White cells	1144	1345	1564	1453	[a]
Columnar epithelial cells	96	97	86	81	[a]
Squamous epithelial cells	258	306	221	259	[a]
Differential counts (%)					
Macrophages/ monocytes	50.1	47.3	36.1	39.1	[b]
Neutrophils	34.8	38.4	47.4	36.6	[c]
Columnar epithelial cells	15.0	14.1	10.8	16.8	[a]
Eosinophils	0.1	0.3	5.5	7.0	[d]
Lymphocytes	0.02	0.03	0.23	0.50	[e]
Cytokine concentrations (pg/ml)					
IL-4	28	26	6	2	[f]
IL-6	359	509	629	594	[f,g]
IL-8	6669	6563	4147	5521	[f,g]
IL-10	4.1	4.8	5.4	0.04	[f]
IL-13	18	20	14	4	[f]
GMCSF	48	39	15	26	[f,g]
Interferon	5.0	5.8	1.9	2.5	[f]
TNF	56	61	3	12	[f]

Note. Symbols in Notes column are: [a] No significant difference between atmospheres or clinical groups by ANOVA. [b] Asthmatic < healthy, $p < .05$; atmosphere not significant. [c] Asthma–atmosphere interaction $p < .1$. [d] Asthmatic > healthy, $p < .001$; atmosphere not significant. [e] Asthmatic > healthy, $p < .01$ by nonparametric test; atmosphere not significant. [f] No difference between atmospheres by nonparametric test with all subjects pooled. [g] No difference in UFP-FA difference between healthy and asthmatic groups, by nonparametric test.

tended to report less symptoms with UFP than with filtered air. Figure 3 illustrates that relationship for the net change in total score. The regression slope (strongly influenced by the 2 subjects with most positive symptom score changes) predicted an increase of about 3 points for an increment of 100,000 in particle count. Regression relationships were not significantly different between healthy and asthmatic subjects.

Blood Pressure

Mean baseline systolic/diastolic blood pressure was 106/69 mm Hg in healthy and 118/76 mm Hg in asthmatic subjects. The

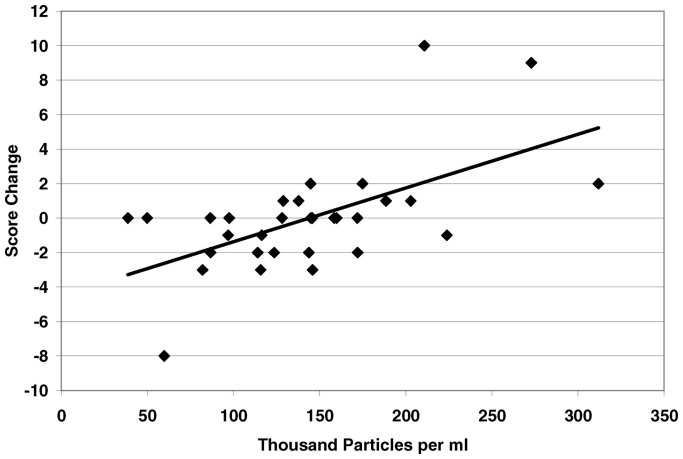

FIG. 3. Individuals' net change of total symptom score during UFP exposure, as a function of their average particle count during exposure (difference: UFP–filtered air). Slope = 0.031 points/(1000 particles/ml); $r^2 = .34$.

healthy-asthmatic difference was significant ($p < .01$). There were no significant changes in blood pressure related to time of measurement or UFP exposure, and no significant relationships were found between net changes in blood pressure and exposure variables.

Holter Electrocardiograms: Analyses of Multihour Periods

Usable Holter recordings were obtained from 16 healthy and 13 asthmatic subjects. Table 6 summarizes their results from measures that assessed complete Holter recording periods 1 (preexposure testing, exposure, and the immediately following 4 h) and 2 (the subsequent 18–20 h). Arithmetic means are shown for variables with relatively normal distributions, and geometric means for those with skewed distributions, which were log-transformed for ANOVA. (If an incidence measurement was zero in a recording period of n hours, incidence was estimated as one event in $2n$ hours.) Unless results differed by asthma status, they are reported for all subjects pooled.

Incidence of ectopic beats was generally low and showed no significant variation by atmosphere, clinical group, or time period. No significant relationships were found between individual ectopic beat incidence and individual measures of UFP exposure. However, the incidence of supraventricular ectopics showed a significant ($p < .05$) positive correlation with ambient PM_{10} concentration at local monitoring stations over the 24 h preceding exposure, when both were expressed as differences UFP–filtered.

Abnormal ST voltage excursions were infrequent or undetectable in most subjects, but occurred several times per hour in a few subjects. Incidence of ST voltage excursions was higher during/after UFP exposures relative to filtered air, to a near-significant degree ($p = .09$ for atmosphere main effect), and

TABLE 5
Significant ($p < .05$) correlations between individual sputum measurements and individual exposure levels

Parameter	Total squamous cells	Percent macrophages/ monocytes	Percent eosinophils	Percent lymphocytes	IL-10	GMCSF
Particle count					All [−.39]	
PM mass				All [+.40]		
Elemental carbon		Healthy [+.64]				
Sulfur				All [+.46]		
				Asthma [+.85]		
Iron	All [+.41]					
Sodium				Asthma [−.68]		All [+.48]
				Healthy [+.61]		Healthy [+.52]
Chamber temperature		Asthma [−.72]	All [−.42]			
Ambient PM_{10}			All [−.40]		All [+.44]	
			Asthma [−.76]			

Note. All measurements expressed as differences UFP – filtered. Sputum variables not listed in table showed no significant correlations with exposure variables, either for all subjects pooled or for healthy or asthmatic groups separately. (IL-4, IL-10, IL-13, interferon, and TNF were not tested in healthy or asthmatic subjects separately because of very low statistical power due to the many undetectable values.)

higher during period 1 than period 2, to a near-significant degree ($p = .08$ for time period main effect). Incidence was significantly ($p < .05$) and positively associated with particle count during UFP exposure, if both were expressed as differences UFP–filtered air, as shown in Figure 4. This relationship was not significantly different between healthy and asthmatic groups. Higher incidence of ST voltage excursions was also positively associated with sodium concentrations in UFP exposures, and with outdoor ambient temperature, but not with exposure chamber temperature.

The SDNN, i.e., the standard deviation of all normal-beat-to-normal-beat intervals (NN) throughout a recording, increased from period 1 to 2 as expected given the longer duration of period 2, but the difference was nonsignificant. A possibly meaningful effect of UFP on SDNN was observed: Healthy subjects' mean decreased with UFP relative to filtered air during period 1 only, while asthmatics' mean decreased during period 2 only, to a near-significant degree ($p = .06$ for interaction of clinical group with time). The SDANN5 (the standard deviation of NN means from successive 5-min intervals throughout a recording) and the ASDNN5 (the mean of NN standard deviations from successive 5-min intervals) showed significant differences between periods 1 and 2, but ANOVA results gave no indication of UFP effects. The ASDNN5 for period 1 showed a significant

TABLE 6
Summary of ANOVA results from multihour Holter electrocardiogram measures

		Filtered		UFP		
Measure	Subjects	Period 1	Period 2	Period 1	Period 2	Note
Ventricular ectopic beats/h, geometric mean	All	0.9	0.9	0.9	0.9	[a]
Supraventricular ectopic beats/h, geometric mean	All	3.5	3.3	3.3	3.4	[a]
ST voltage excursions/h, geometric mean	All	1.5	0.7	1.6	1.3	[b]
SDNN (ms), mean	Healthy	147	151	137	151	[c]
	asthma	136	148	136	140	
SDANN5 (ms), mean	All	110	127	107	121	[d]
ASDNN5 (ms), mean	All	80	73	76	74	[d]

Note. Symbols in Notes column: [a] No significant variation by atmosphere or time period. [b] Atmosphere difference near significance ($p = .09$); time period difference near significance ($p = .08$). [c] Interaction of atmosphere, time period, and asthma status near significance ($p = .06$). [d] Time period difference significant, $p < .05$.

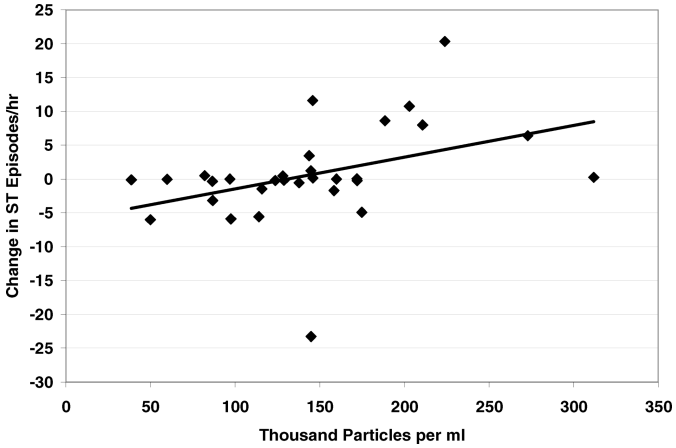

FIG. 4. Individuals' incidence of abnormal S-T voltage excursions during Holter ECG recording period 1 (including exposure and first 4 h afterward), as a function of their average particle count during exposure. Both variables are expressed as difference: UFP – filtered air. Slope = (0.047 events/h)/(1000 particles/ml); $r^2 = .16$.

($p < .05$) negative relationship with sodium concentration in UFP exposures. No other significant exposure-response relationship was found for any measure of heart rate variability in multihour recordings.

Holter Electrocardiograms: Analyses of 5-Minute Quiet Rest Periods

Table 7 summarizes results from Holter measurements during the last 5 min of 10-min quiet rest periods in each testing session, for all subjects pooled. Mean NN showed significant ($p < .001$) variation over time, with a decrease of about 8% at hour 4 relative to preexposure. There was no significant net change with UFP relative to filtered air. The decrease in NN (increase in heart rate) at hour 4 was reversed by day 2 in healthy subjects, but persisted in asthmatics (significant interaction of time and clinical status, $p < .05$). Low-frequency (LF) power, associated with sympathetic nervous system influence on the heart, showed significant ($p < .05$) variation related to UFP exposure, with an appreciable negative net change at hour 4, reversed by day 2, not significantly influenced by asthma status. The net change at hour 4 showed a significant ($p < .05$) negative relationship with particle count. The regression slope predicted a loss in log-transformed LF of 0.33, representing a 53% decline in geometric mean LF power, for an increment of 100,000 particles/ml. The postexposure net change related positively to sodium concentration, and the day 2 net change related negatively to chamber temperature. High-frequency (HF) power, associated with parasympathetic influence on the heart, showed no significant overall difference between UFP and filtered air, although its net change on day 2 increased with iron concentration. Total power likewise showed no significant overall difference between atmospheres. Its net change at hour 4 related negatively with elemental carbon concentration, and its net change on day 2 related negatively with chamber temperature.

TABLE 7
Summary of ANOVA results from Holter ECG measurements during 5-min quiet rest periods

Measure	Mean baseline	Mean net change			Note
		Post	Hour 4	Day 2	
NN (ms)	930	−7	+11	+10	[a]
SDNN (ms)	72	−4	−7	+3	[b]
Log LF power	0.68	−0.05	−0.19	+0.09	[c,d]
Log HF power	0.56	−0.08	+0.10	+0.02	[b,d]
Log Total power	1.34	−0.12	−0.16	+0.06	[b,d]

Note. Symbols in Notes column: [a] No significant variation in net change of NN; significant variation of NN itself is described in text. [b] No significant variation. [c] Significant variation in net change over time, $p < .05$. [d] Low-frequency (LF) "sympathetic" power is measured between 0.04 and 0.15 Hz. High-frequency (HF) "parasympathetic" power is measured between 0.15 and 0.40 Hz. Units of power before log transformation are $(\text{beats/min})^2$.

Hematology

Red blood cell counts averaged near 4.5 million/μl overall. They tended to fall slightly after exposure, more so with UFP than with filtered air. The overall mean net loss attributable to UFP, 88000/μl ($p = .04$), did not vary significantly by time or asthma status. The overall mean white cell count was near 6000/μl. Counts were higher both before and after UFP exposures, relative to filtered air ($p < .01$ for atmosphere main effect). There was an increase from preexposure to hour 4 averaging near 500/μl, followed by a larger decrease from hour 4 to day 2 ($p < .005$ for time effect), not significantly different between UFP and filtered air. Neutrophil, lymphocyte, and monocyte counts showed this circadian pattern. Eosinophils were higher in asthmatic than healthy subjects ($p < .01$), but showed no significant changes related to exposure. Basophils showed no significant variation in ANOVA. Net changes in white cell counts were not significant. Platelet counts averaged 245/μl overall. Like white counts, they were significantly ($p < .01$) higher with UFP than with filtered air before and after exposure. Platelets' net change varied significantly ($p = .02$) with time, averaging –3 at hour 4 and +4 at day 2. Net changes were larger in asthmatic than healthy subjects, but the difference did not reach significance. Correlations of hematologic net changes with exposure differences were significant no more often than expected by chance, and no meaningful patterns were found.

Blood Biochemistry

Blood biochemistry measurements relevant to coagulation factors, fibrinolysis, endothelial activation, and systemic

TABLE 8
Summary of ANOVA results from blood biochemistry measurements

Assay[a]	Units	n[b]	Baseline[c]	Mean net change			p Value	
				Post	Hour 4	Day 2	Mean	Time
D-dimer	ng/ml	4 H,10 A	244	−36	−65	−84	.01	.31
vWF	%norm.	4 H,10 A	154	−14	−16	−11	.32	.92
PAI-1	ng/ml	17 H,12 A	25.4	+3.2	+1.2	+0.7	.17	.59
CRP	μg/ml	17 H,12 A	6.61	+5.40	+2.42	−0.01	.10	.70
Factor VII	ng/ml	4 H,10 A	177	−19	+4	−5	.39	.35
Factor IX	μg/ml	10 A	3.21	−0.20	−0.35	−0.52	.03	.16
		4 H	3.23	+0.05	−0.07	+0.40	.80	.07
Fibrinogen	mg/ml	17 H,12 A	2.01	−0.33	−0.14	−0.25	.46	.79
Plasminogen	mg/ml	17 H,12 A	0.89	+0.47	+0.11	+0.08	.32	.35
TPA	ng/ml	17 H,12 A	7.08	+0.22	+0.25	−0.18	.84	.92
sICAM-1	ng/ml	13 H,2 A	86.8	+9.7	+3.1	+11.7	.53	.80
sVCAM-1	ng/ml	13 H,2 A	856	+57	+16	+45	.65	.94
MPO	pg/ml	13 H,2 A	4156	−55	+453	+1008	.56	.42
e-Selectin	ng/ml	13 H,2 A	33.9	+2.9	+4.2	+8.3	.31	.50
CD40 ligand	pg/ml	13 H,2 A	352	+155	+179	+37	.26	.32

Note. Only the D-dimer summary line and the first Factor IX summary line show significant difference by p value.

[a] vWF, vonWillebrand factor; PAI-1, plasminogen activator inhibitor 1; CRP, C-reactive protein; TPA, tissue plasminogen activator; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular adhesion molecule 1; MPO, myeloperoxidase.

[b] Number of subjects with usable data; H = healthy, A = asthmatic.

[c] Mean before filtered air study.

inflammation were available for between 14 and 29 subjects, depending on the particular assay. Table 8 summarizes ANOVA results. For the two factors that showed significant UFP-related variation, data were available for only 14 subjects, 10 of them asthmatic. D-dimer showed a significant negative net change overall, not significantly different among the 3 times of measurement after exposure. Factor IX showed an asthma–time interaction, the only significant asthma effect found in blood biochemistry data. Mean factor IX fell progressively in the 10 asthmatics after UFP relative to filtered air, while in the 4 healthy subjects it remained stable through hour 4 but rose on day 2. None of the other 12 quantifiable factors showed any significant variation. (Interleukin-6, not tabulated, was usually below quantifiable limits.) Correlations of net biochemical change with exposure difference were calculated for the variables with data available for 29 subjects. Increased particle count was significantly related to increased C-reactive protein, plasminogen, and tissue plasminogen activator (TPA) immediately postexposure, and to increased TPA at hour 4. These relationships appeared to be driven by large net increases in the two subjects with exposure particle counts >250,000/ml. Rank correlations with particle count were nonsignificant for these and all other blood biochemical variables. However, particle mass concentration in UFP exposure showed significant rank correlations with C-reactive protein net change at all three times of measurement (negative), and with plasminogen and TPA at hour 4 (positive). Except for plasminogen, these same variables showed significant rank correlations with prior 24-h ambient PM_{10}, of opposite sign.

DISCUSSION

In this study as in our previous studies of larger particles collected from the same location, we report statistically significant effects of ultrafine particles. Yet, consistent with our previous studies, these observed effects were generally small and without any obvious pattern. Thus, they provide only limited, equivocal corroboration of the extensive epidemiologic evidence for adverse health effects of urban ambient particles.

The most statistically significant negative effect of UFP we found was a decrease in SpO_2. We also observed SpO_2 decreases (albeit with a different time course) in elderly adults exposed to concentrated fine particles (Gong et al., 2004a), but not in young and middle-aged adults exposed to fine or coarse particles (Gong et al., 2003a, b, 2004b). The mean decrease in SpO_2 in this study was clinically trivial—smaller than the limit of resolution in an individual test—and not obviously related to UFP dose, but it raises the possibility of impaired oxygen delivery in susceptible individuals with preexisting decrements of arterial oxygen tension. Other respiratory tests do not offer a clear explanation for reduced SpO_2. The apparent slight loss in FEV_1 was delayed relative to the SpO_2 loss, especially in the healthy cohort. No evidence of modified airway inflammation after UFP exposure was found in induced sputum cells, nor was there a clear change

in eNO in either subject cohort that could have reflected airway dysfunction leading to slight impairment in oxygen delivery.

UFP exposures had no detectable effect on the incidence of ectopic heartbeats, although increasing ambient pollution as measured by the prior 24-h mean PM_{10} was associated with increasing incidence of supraventricular ectopic beats. The incidence of abnormal ST voltage excursions suggesting changes in myocardial repolarization showed a possible influence of experimental UFP exposure, in terms of a near-significant overall difference between UFP and filtered air conditions, and a significant tendency for individuals with higher particle counts in their exposures to experience more excursions. Multihour SDNN measurements suggested a reduction in heart rate variability during and/or after UFP exposure, which fell short of statistical significance. Somewhat stronger evidence for reduced heart rate variability—a frequent finding in epidemiologic panel studies—was provided by low-frequency (sympathetic) power measurements during quiet rest 4 h after UFP exposure ended. Both the overall net change with UFP relative to filtered air, and the regression slope of individual net change versus individual particle count during UFP exposure, were statistically significant.

Relative to the Chapel Hill UFP study (Samet et al., 2007), our exposures had similar mean particle count but higher mean particle mass concentration. Given that ours is a much larger and more polluted metropolitan area, our subjects likely experienced higher prestudy and intercurrent ambient PM exposures than the Chapel Hill subjects. (For example, in 2005 annual mean $PM_{2.5}$ exceeded 22 $\mu g/m^3$ in Los Angeles County, compared to 13–14 in the Chapel Hill area [http://www.epa.gov/air/data/].) This might tend to reduce their susceptibility to acute effects. On the other hand, about half our subjects had risk factors (asthma and/or age 35–50) excluded from the Chapel Hill volunteer group, which might make our subjects more susceptible. Despite these differences, many results appear consistent between the two studies: little or no meaningful change in conventional pulmonary function tests, little evidence of airway inflammation (assessed by sputum induction here, by bronchoalveolar lavage in Chapel Hill), a tendency for UFP exposure to decrease some measures of heart-rate variability, and a lack of significant change in most blood coagulation factors and systemic-inflammation markers. Isolated significant changes in D-dimer were observed in both studies, but here data were available for fewer than half the subjects, and the observed change was negative, whereas it was positive in Chapel Hill.

In summary, of the many cardiac, pulmonary, and blood tests undertaken to examine acute effects of ultrafine particle exposure, few showed changes beyond what could be expected by chance. Of these few, most were deleterious, but comparatively small—too small to be considered clinically significant if observed in an individual. The same can be said for other human studies of concentrated ambient particles. Thus, the goal of strong coherence between human toxicologic and epidemiologic evidence of the negative health effects of particles remains elusive. It may be useful to consider the limitations of controlled-exposure and epidemiologic studies that contribute to this problem. On the epidemiologic side, there may have been too little attention to weather factors, apart from temperature, that could influence both PM pollution and cardiopulmonary stress, and thus act as confounders or effect modifiers in analysis of PM–health relationships. In controlled-exposure studies, small nonrepresentative samples are an obvious inherent limitation, exacerbated by diverse exposure and monitoring techniques that make it difficult to pool data from multiple studies for statistical analysis. Another issue is the limited ability to control intercurrent ambient exposures—a larger problem with PM than with ozone or sulfur dioxide exposures, for which laboratory studies have provided clearer evidence of toxicity at near-ambient levels. A typical subject's inhaled dose of ambient particles might be increased by no more than a factor of 2 on an experimental PM exposure day, and reduced by no more than 10% on a filtered-air control day. Still another challenge is the combination of secondary stresses (confinement, noise, lack of precise temperature control) inherent in particle-concentrator-based exposure protocols, which might mask subtle responses to PM. Future studies will require further efforts to overcome these problems.

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